Public Health Goal for Methoxychlor In Drinking Water

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based solely on health effects without regard to cost impacts and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR METHOXYCHLOR IN DRINKING WATER

SUMMARY

A Public Health Goal (PHG) of 0.03 mg/L (30 ppb) has been developed for the pesticide methoxychlor in drinking water. The existing California Maximum Contaminant Level (MCL) is 0.04 mg/L (40 ppb). Methoxychlor has low acute toxicity to mammals. Its pesticidal mechanism of action is apparently identical to that of DDT, and, like DDT and DDE, methoxychlor has weak estrogenic activity. Estrogenic effects of methoxychlor are mediated through its demethylated metabolites. The methoxychlor PHG is based on the lowest-observed-adverse-effect level of 5 mg/kg-day for effects on the reproductive system in female rats exposed during the perinatal period, and a combined uncertainty factor of 1000. The effects at 5 mg/kg-day included delayed vaginal opening, decreased ovary weight, lower FSH levels during estrus, and decreased weight of the pregnant uterus. These data are supported by similar LOAELs and NOAELs in other reproductive and developmental studies, chronic animal studies, and by a single subacute study in adult human males and females. Although several related organochlorinated pesticides are classed as carcinogens, cancer bioassays of methoxychlor have been negative. In vivo metabolism of methoxychlor is relatively rapid, so it does not bioaccumulate as do many other halogenated hydrocarbon pesticides. It is also only moderately persistent in the environment, and is rarely found in air, soil, or water except near sites of production or disposal. Suspension of pesticidal usage of methoxychlor in California in 1995 means that exposures from environmental media in California should be decreasing, although it is still found in food grown in other states or countries. Sensitive populations and sensitive developmental periods, and exposure to other chemicals with estrogenic activity have been considered in calculating the health protective concentration for methoxychlor in drinking water.

INTRODUCTION

The purpose of this document is to describe the development of a PHG for the insecticide methoxychlor (Marlate®) in drinking water. This DDT analog was very heavily used after cancellation of DDT in the 1970s, although usage has declined more recently. In the early '90s about 300 to 500 thousand pounds of methoxychlor was used per year in the US (ATSDR, 1994). Compared to DDT, methoxychlor is rapidly metabolized both in the environment and in living organisms, so it does not produce the long-lasting toxicity and bioaccumulation, which led to the cancellation of DDT. However, use of methoxychlor was suspended in California in late 1995 due to deficiencies in its toxicity study database.[1] Methoxychlor use in California appears to have decreased greatly before its suspension, with only 1188 pounds of active ingredient reported used in agriculture in 1995 (DPR, 1996a).

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[1] Ortho Home Orchard Spray remains listed as active in the online pesticide database maintained by the California Department of Pesticide Registration (DPR). This status was maintained to allow leftover materials to be used up, according to DPR. Methoxychlor is no longer being sold.

The potential for detectable concentrations of methoxychlor in California drinking water has undoubtedly decreased greatly since its suspension, although methoxychlor is still available in other regions of the country. It is or has been used against insects on fruit and shade trees, vegetables, dairy and beef cattle, in home gardens, commercial greenhouses, and landscape maintenance, for food storage and seed pretreatment, and in public health insect control. It has often been formulated with other pesticide products.

A Maximum Contaminant Level (MCL) of 40 ppb (0.04 mg/L) was established by the California Department of Health Services (DHS) (CCR Title 22, Section 64444) in 1977. This level is identical to the federal MCL of 0.04 mg/L for methoxychlor (U.S. EPA, 1991). Methoxychlor is not listed under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as a chemical known to the state to cause cancer or reproductive toxicity. U.S. EPA has judged methoxychlor not classifiable as to its carcinogenic potential (U.S. EPA 1998).

In this document, available data on the toxicity of methoxychlor are evaluated, particularly in the context of recent perspectives on environmental estrogens. The U.S. EPA's reproductive toxicity risk assessment guidelines (U.S. EPA, 1996) were also considered. To determine a public health-protective level of methoxychlor in drinking water, relevant studies were identified, reviewed and evaluated, and sensitive developmental periods and human subpopulations were considered.

CHEMICAL PROFILE

Chemical Identity

Methoxychlor, 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane, is a bicyclic aromatic chemical related to DDT. The structure is shown in Figure 1 below.

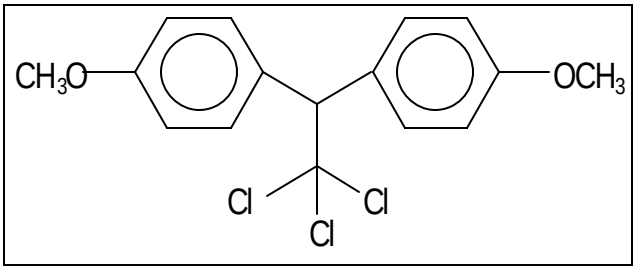

Figure 1. Chemical structure of methoxychlor

In the synthesis of this chemical, many impurities are produced. Earlier versions were only about 50% pure; more recently, the technical grade has been improved to about 88-90% purity (ATSDR, 1994). Some of the congeners may have greater toxicity and environmental persistence than methoxychlor.

Physical and Chemical Properties

Important physical and chemical properties of methoxychlor are given in Table 1. Like other halogenated aromatics, methoxychlor is lipophilic, only slightly soluble in water and is poorly volatile. It binds rather tightly to soil. The slow volatilization and distribution around the globe that has been documented for other halogenated hydrocarbons (Wania and Mackay, 1996) is less of a problem for methoxychlor because of its short environmental half-life (see Environmental Occurrence and Human Exposure).

Table 1. Physical and Chemical Properties of Methoxychlor

Property	Value (ATSDR, 1994)
Molecular weight	345.65
Color	Pale yellow
Physical state	Crystalline solid
Odor	Slightly fruity or musty
Odor threshold	No data
Melting point	89°C (pure), 77°C (technical grade)
Boiling point	Decomposes
Solubility	
Water	0.045 mg/L
Organic solvents	Soluble in aromatics, ketones, aliphatics, alcohols
Density	1.41 g/cm^3
Partition coefficients	
Log K_{ow}	4.7-5.1
Log K_{oc}	4.9
Vapor pressure (25°C)	1.4 x 10^{-6} mm Hg (est.)
Henry's law constant	1.6 x 10^{-5} atm-m^3/mol (est.)
Conversion factors	1 ppm = 14.14 mg/m^3

Production and Uses

This organohalogenated pesticide has been heavily used since the cancellation of DDT in the 1970s, with peak U.S. production in the late '70s to early '80s of over 5 million pounds. Recent usage has decreased; it was estimated to be about 500,000 to 900,000 pounds in 1986, and about

300,000 to 400,000 pounds per year in 1990 to 1991 (ATSDR, 1994). Suspension of methoxychlor in California (December 26, 1995) has presumably contributed to the overall decline in production and use. However, use of methoxychlor in California appears to have tapered off before its suspension, because the annual pesticide use report published by the California Department of Pesticide Regulation (DPR) shows only 1188 pounds of active ingredient used in 1995, the last year for which information is available (DPR, 1996a).

The potential for detectable concentrations of methoxychlor in California drinking water has undoubtedly decreased greatly since its uses were suspended, although methoxychlor is still available in other regions of the country. Methoxychlor has been registered by the U.S. EPA, either alone or in combination with other pesticides, for use against houseflies, mosquitoes, cockroaches, chiggers, various arthropods found on field crops, and insect pests in stored grain or seed for planting. It has been registered for use on more than 85 crops, including fruits, vegetables, soybeans, nuts, and alfalfa. It is also approved for use on forests, ornamental plants, and for insect control around houses, barns, and other agricultural premises (ATSDR, 1994). It has often been formulated with other pesticide products, such as captan, diazinon, and malathion. It has been available in many forms, including technical-grade concentrate, wettable powders, dusts, granules, emulsifiable concentrates, and pressurized sprays for home use.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Most of the introduction of methoxychlor to air would occur during and after its use as a pesticide. Because it has a low vapor pressure and binds well to soil, air concentrations would be expected to be quite low except in the immediate area of a spray application. The photo-oxidation half-life of methoxychlor in air is not known, but has been estimated at 1-11 hours (Howard, 1991). Some long-distance transport of methoxychlor in air, perhaps bound to fine particles, is indicated by the detection of methoxychlor in arctic snow (Welch et al., 1991). However, its lower stability compared to other organochlorinated pesticides makes it less likely to become a global problem.

A survey of pesticide levels in air in two U.S. cities found mean levels of methoxychlor of 0 to 100 picogram/m^3 in outdoor air and 200 to 300 pg/m^3 in indoor air (U.S. EPA, 1990a). In a Canadian study, the yearly mean level of methoxychlor in air was reported to be 1.7 pg/m^3. Levels were higher during insect control periods (up to 27 pg/m^3) and generally below the detection limit (about 0.1 pg/m^3) during non-use time periods (Hoff et al., 1992). Levels of methoxychlor in California air would thus be expected to be extremely low.

Soil

Methoxychlor is degradable in soil to less hydrophobic compounds, both under aerobic and anaerobic conditions. Anaerobic biodegradation half-life was reported to be less than 30 days, while aerobic biodegradation half-life was greater than 100 days (Muir and Yarechewski, 1984). Residues were detectable in soil at least 18 months after soil treatment (Golovleva et al., 1984). The major environmental degradation pathways involved dechlorination and demethylation. The extent to which the degradation products may accumulate in soil is not clear, although some bacterial strains can extensively metabolize the pesticide. It should be noted that the

demethylated products have estrogenic activity (Cummins, 1997). Intact methoxychlor binds tightly to soil, and will be found in the top few inches after agricultural applications. The metabolites, being more polar, can migrate in soil (Golovleva et al., 1984). Migration of methoxychlor bound to sediment particles is also possible.

Water

Methoxychlor has been occasionally detected in surface waters at low levels, ranging from 0.032 to 15 ng/L (ATSDR, 1994). It was not found, however, in domestic or municipal drinking water supplies in several surveys in various regions of the country (U.S. EPA, 1990a,b). Methoxychlor has been found in surface waters near points of application for pest control, and in groundwater near waste disposal sites (ATSDR, 1994). Methoxychlor in surface waters would be distributed mostly in the sediment fraction due to its low water solubility and tight binding to lipophilic sites on soil particles. Overall, water would be expected to be a minor source of exposure to methoxychlor for California residents.

Food

Residues of methoxychlor have been found in a small proportion of food samples of various types, including vegetables, fruits, and grains. Bioconcentration can occur, dependent on the rate of metabolism of methoxychlor. Bioconcentration factors of about 100 to 8000 have been reported in several fish species. Occasional samples of fish from the Great Lakes had detectable methoxychlor, with some tissue levels as high as 100 ppb (ATSDR, 1994). In the U.S. FDA's Total Diet Study in 1995, methoxychlor was detected at low levels in less than 3% of the samples (which was below the cutoff point for specific discussion of incidence and levels) (U.S. FDA, 1996). Methoxychlor was not reported present in produce sampled in the 1994 California pesticide monitoring program (DPR, 1996b). Methoxychlor can be excreted in a biologically active form in milk, which could be relevant for infants and children (Ivey et al., 1983; Appel and Eroschenko, 1992; Chapin et al., 1997). Average daily intake values for methoxychlor in food were calculated as 0.001 to 0.006 µg/kg-day by Gunderson (1988) based on the FDA's monitoring for the total diet study in 1982-1984. Infants' daily dietary intake was estimated to average 0.019 µg/kg-day from the FDA's 1980-1982 data. Current exposures are expected to be lower, based on the substantial decrease in agricultural usage and its moderate environmental persistence. However, it is reasonable to conclude that foods, including fish, would be the major methoxychlor exposure source for California residents.

Other Sources

There should be no significant occupational exposures to methoxychlor in California, since the pesticide has been suspended from use. Similarly, exposures during home use in gardens and orchards, as well as for flea and insect control, should be decreasing as home supplies are used up. The major exposure source for California residents should now be methoxychlor in food, from commodities grown out of state.

METABOLISM AND PHARMACOKINETICS

Absorption

Oral absorption of methoxychlor is apparently quite efficient, although no specific estimates are available for humans. Oral absorption in mice has been estimated to exceed 90% (ATSDR, 1994). For this assessment, oral absorption is assumed to be equivalent in humans and experimental animals. Dermal absorption of methoxychlor deposited directly on skin would be expected to be low and slow. Limited studies in goats and cows have demonstrated a low degree of systemic absorption after dermal applications (Skaare et al., 1982; Davison et al., 1983; Ivey et al., 1983). Absorption should be similar to DDT, for which dermal penetration has been measured as 9 to 30% in rhesus monkeys after the pesticide was applied in acetone (Wester et al., 1990). Data on inhalation absorption of methoxychlor are not available. We assume that pulmonary absorption of methoxychlor would be essentially complete, i.e., equivalent to the inspired volume minus dead space, or about 70% in humans.

Distribution

Methoxychlor is distributed throughout the body, and because of its lipophilicity is taken up very well into fat. Having a log K_{ow} of 4.7 to 5.1, at equilibrium it would be about 100,000 times higher concentration in a lipid phase than in a contiguous aqueous phase. In actual tissues the partitioning is not this extreme because of lipids in blood and water in fatty tissues. With repeated daily administration, fat and liver levels reach an equilibrium, then appear to decrease, possibly because of induction of metabolism. Residual levels decline rapidly after feeding stops, so that methoxychlor levels approach the detection limit within a few days (ATSDR, 1994). Methoxychlor crosses the blood-brain barrier and the placenta, and also partitions into the lipids of milk (Ivey et al., 1983; Swartz and Corkern, 1992; Appel and Eroschenko, 1992; Cummings, 1997; U.S. EPA, 1998). The metabolites are secreted into the bile. The extent of enterohepatic circulation is unknown, but the metabolites are mainly excreted in the feces.

Metabolism

Methoxychlor is metabolized by cytochrome P_{450} isozymes in liver in both rodents and humans, to produce mono- and bis-hydroxy O-demethylated (phenolic) metabolites (Li et al., 1995; Dehal and Kupfer, 1994; Kupfer et al., 1990). This is a NADPH-requiring, phenobarbital-inducible reaction. Dehydrochlorination occurs concurrently, so that a mixture of demethylated, dehydrochlorinated products is formed. These major mammalian metabolic pathways are summarized in Figure 2. Subsequent metabolic reactions may include ring hydroxylation in the meta positions, complete dechlorination, and various conjugation reactions to form more hydrophilic products. The metabolic products are secreted in the bile, presumably in the form of conjugates, and are ultimately excreted in feces.

The actual mixture of reaction products found in vivo (and its net estrogenic activity) is complicated by the presence of several different impurities in the technical-grade methoxychlor (Kupfer and Bulger, 1987).

Because the liver O-demethylation reactions are relatively rapid, methoxychlor does not accumulate in the body like its analog, DDT (p,p'-dichlorodiphenyltrichloroethane). The more water-soluble phenolic metabolites of methoxychlor are readily metabolized further and excreted. In addition, the inducibility of the cytochrome P_{450} metabolizing enzymes at higher doses of methoxychlor should be considered. With induction of metabolism, any chronic toxic effects due to the intact chemical should be lessened, while estrogenic effects, which are largely due to the phenolic metabolites, could be enhanced.

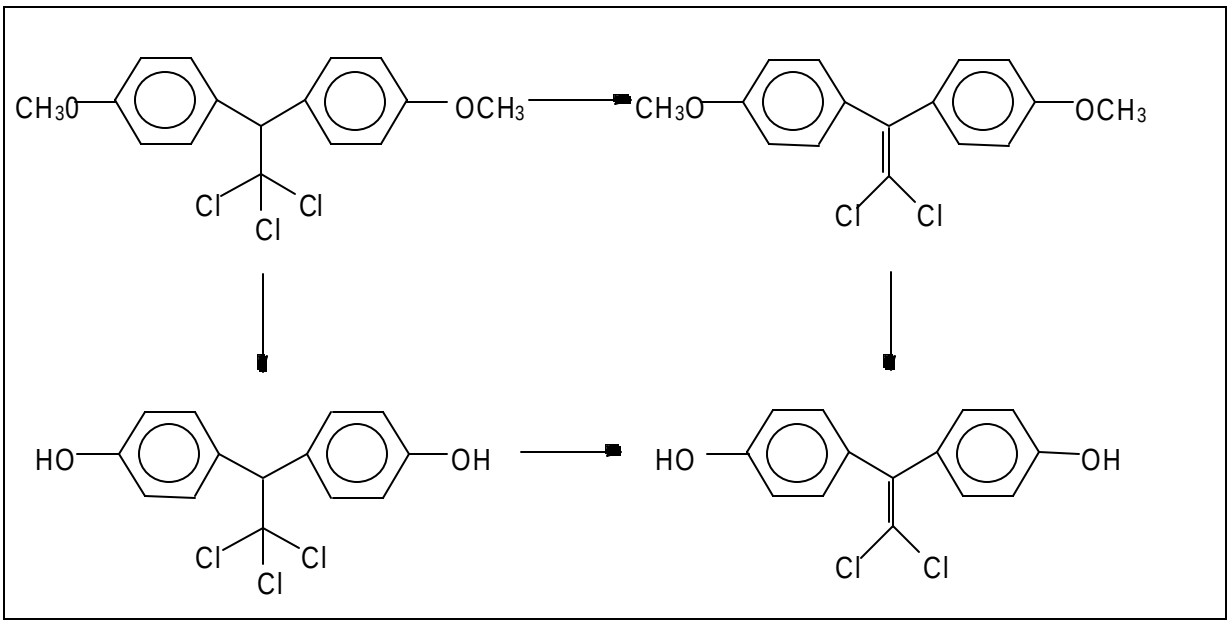

Figure 2. Major in vivo metabolic pathways for methoxychlor.

Excretion

Methoxychlor was about 90% excreted into the feces in the form of metabolites in mice; the other 10% was excreted in the urine (Kapoor et al., 1970). In a lactating female, however, a fraction of the total intake is secreted into milk in the form of both intact methoxychlor and phenolic metabolites. For highly lipophilic chemicals with very long half-lives, incorporation into milk can represent a significant excretion pathway, and a correspondingly high exposure pathway for babies drinking the milk. This has much less significance for methoxychlor because it is rapidly lost from the body by other pathways.

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

High doses of methoxychlor cause tremors, convulsions, and other signs of neurological stimulation. These acute effects are similar to DDT and the Type 1 pyrethroids. Acute oral LD_{50}s are generally greater than 3000 mg/kg in mammals and 2000 mg/kg in birds for technical-grade methoxychlor. More purified preparations tend to be less toxic, with rat LD_{50}s greater than 5 g/kg (Cummings, 1997). However, fish and aquatic invertebrates are quite sensitive to methoxychlor. The 96-hr LC_{50}s for fresh and salt-water fish range from about 0.006 to 0.1 ppm, while the LC_{50}s for various aquatic invertebrates are as low as 0.001 ppm (Sax, 1987; U.S. EPA, 1988).

No acute toxic effects are reported on the reproductive system in mammals, which is the critical site for subchronic and chronic effects.

Subchronic Toxicity

With repeated dosing, reproductive effects become apparent in both males and females. These appear to be mediated largely by the phenolic metabolites of methoxychlor, which bind much more efficiently to estrogen receptors than does the intact chemical (Cummings, 1997). These effects are discussed below. However, it is not clear whether all the subchronic effects of methoxychlor are mediated through direct actions on reproductive organs. Effects on the hypothalamic-pituitary axis of male rats have also been noted at low doses (25 and 50 mg/kg-day for 56 days), which may be a direct effect rather than mediated through a testicular feedback loop (Goldman et al., 1986). Methoxychlor may also alter some hormonal systems through alteration of liver metabolism, such as metabolism of thyroid hormones, although it does not appear to be a strong liver enzyme inducer (Zhou et al., 1995).

Genetic Toxicity

Methoxychlor has been found to be negative in several Ames assay mutagenicity tests (Simmon, 1979; Probst et al., 1981; Waters et al., 1982) with and without metabolic activation. Unscheduled DNA synthesis assays in rat hepatocytes and human fibroblasts were also negative, as was the Drosophila sex-linked recessive lethal assay (Simmon, 1979; Probst et al., 1981; Waters et al., 1982). Negative results have been reported in a transformation assay in rat embryo cells (Dunkel et al., 1981; Traul et al., 1981), in Syrian hamster embryos (Dunkel et al., 1981), and in Chinese hamster ovary cells (Oberly et al., 1993). A positive result has, however, recently been reported for the induction of forward mutations in the mouse lymphoma assay (Oberly et al., 1993). In addition, positive results have been reported in the mouse lymphoma cell mutagenesis assay (Mitchell et al., 1988; Myhr and Caspary, 1988).

Formation of protein adducts has been reported in methoxychlor metabolism studies in rat liver. These covalently bound liver microsomal protein adducts appear to be formed in the cytochrome P_{450}-mediated metabolism of methoxychlor, although the mechanism and significance is not entirely clear (Bulger et al., 1983; Bulger and Kupfer, 1990). Formation of DNA adducts has not been reported. Methoxychlor does not induce DNA breaks in human or rat testicular cells in an in vitro DNA-damage assay (Bjorge et al., 1996).

Developmental and Reproductive Toxicity

Reproductive effects of methoxychlor have been extensively studied in animals. The reproductive system is a sensitive target of methoxychlor toxicity in both males and females. Effects include histopathological changes in the reproductive organs and accessory glands, disrupted sexual maturation and reproductive function, altered hormone levels, and changes in a wide variety of endocrine-related parameters, such as sexual behaviors. These effects are caused by the estrogenic activity of both the O-demethylated metabolites of methoxychlor and some of the O-demethylated contaminants of technical grade methoxychlor; intact methoxychlor has little affinity for the estrogen receptors (Bulger et al., 1985; Cummings, 1997). Methoxychlor is not listed as a reproductive toxicant under California's Proposition 65. Information regarding the reproductive effects of methoxychlor in male and female animals is presented separately below. Some additional perspective on developmental or reproductive effects after long-term exposures is provided in the chronic toxicity section.

Reproductive Effects in Female Animals

Methoxychlor affects development of the female reproductive system. The most sensitive effects have been observed with perinatal treatments. This is presumably a time of high sensitivity because of the rapid development of the reproductive system during this period.

Chapin et al. (1997) gavaged Tac:N(SD)fBR rats with 95% methoxychlor in corn oil from gestational day 7 to postnatal day 7 at doses of 0, 5, 50, or 150 mg/kg-day. The pups were then individually dosed by gavage from postnatal day 7 to day 42. One set of offspring was allowed to mate at 12 weeks of age and the females were killed for examination at gestational day 18. Extensive gross, histopathological, chemical, and behavioral measurements were made on both male and female treated offspring. Adverse effects were seen on multiple reproductive system parameters in both sexes. The most significant low-dose effects were seen in females, including LOAELs for delayed vaginal opening and decreased ovary weight at postnatal day 46 of 5 mg/kg, the lowest dose tested. Decreased weight of the empty uterus at day 18 of pregnancy and lowered FSH levels during estrus were also observed in adult female rats after the perinatal treatments with methoxychlor at 5 mg/kg-day and higher. These effects are tabulated in Table 2.

Table 2. The critical sensitive effects of methoxychlor in female rats (Chapin et al., 1997)

EFFECT	DOSE, mg/kg-d			
	Control	5	50	150
Age at vaginal opening (days ±SE)	37.4±0.6	35.2±0.5*	30.8±0.2*	33.4±0.3*
Decreased ovary weight at postnatal day 46 (g ±SE, % of control)	0.065±0.004 (100%)	0.047±0.003* (72%)	0.030±0.043* (46%)	0.03±0.008* (46%)
Weight of empty uterus at gestation day 18 (g ±SE, % of control)	5.13±0.20 (100%)	4.06±0.30* (79%)	2.51±0.60* (49%)	None pregnant
Serum FSH levels during estrus (log of ng/ml ±SE, % of control)	0.79±0.03 (100%)	0.57±0.05* (72%)	0.33±0.04* (42%)	NR

*p <0.05; NR = not reported

These results are also represented in graphs to help evaluate the dose response. Age at vaginal opening is biphasic, as shown in Figure 3, with the 5 mg/kg-day appearing to be close to a threshold for a decrease in the age, and 50 mg/kg-day a possible maximum.

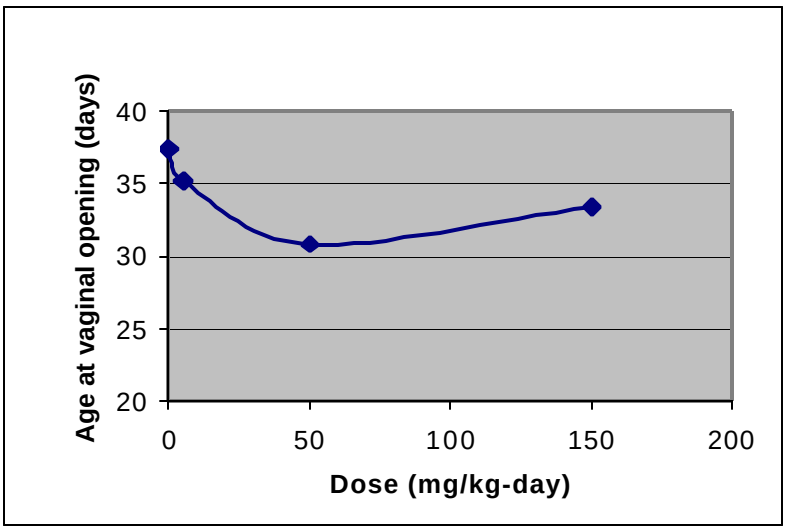

Figure 3. Effect of methoxychlor on age at vaginal opening (Chapin et al., 1997)

Figure 4 shows the effects of methoxychlor on ovary weight at postnatal day 46 after the perinatal treatments to dam and offspring. These data are plotted with dose on a logarithmic scale to show the log-linear extrapolation of effect to low levels. On this scale, the effect would appear to extrapolate to a 10% decrease from control levels (a common level for benchmark extrapolation methods) at about 1 mg/kg-day. The zero effect level (0.065 g) would correspond to about 0.5 mg/kg.

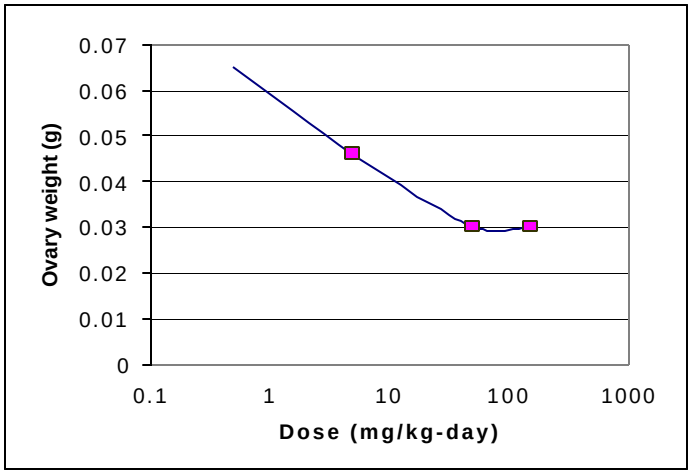

Figure 4. Effect of methoxychlor on ovary weight (Chapin et al., 1997).

Figure 5 shows the weights of the empty uteri on day 18 of pregnancy and the FSH levels during estrus in adult female rats who were perinatally treated with methoxychlor. These values are plotted using a linear dose-response function. It could be considered that the effects at 50 mg/kg-day (the largest dose plotted) are near the maxima for these functions because estrous cycles were interrupted at the next higher dose (150 mg/kg-day), and none of the females became pregnant. When plotted on a logarithmic dose scale, a straight-line extrapolation through 5 and 50 mg/kg-day passes through zero effect at 0.5 to 1 mg/kg-day.

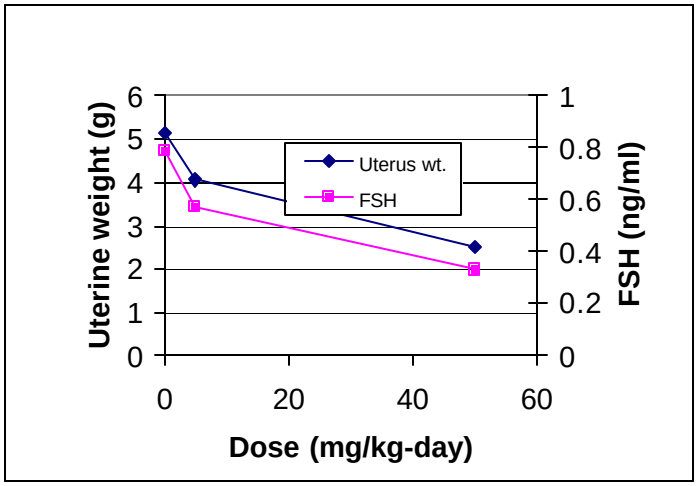

Figure 5. Weight of pregnant uterus and FSH levels during estrus (Chapin et al., 1997).

The male rats showed less sensitivity to methoxychlor than the females. Only one measured parameter, the seminal vesicle weight, was significantly decreased at 5 mg/kg-day, and this did not exhibit a dose-response function (no significant effect at 50 mg/kg). Females receiving the highest-dose perinatal treatment of 150 mg/kg-day failed to produce litters when mated as adults (0/15), while at the 50 mg/kg-day dose, only 3/15 delivered live litters.

In summary, the Chapin et al. (1997) data appear to show a sensitive effect on reproductive parameters with a LOAEL of 5 mg/kg-day, and an extrapolated NOAEL of about 0.5 to 1 mg/kg-day. For risk assessment, we will utilize the default uncertainty factor of 10 to estimate the NOAEL from the LOAEL for this study, i.e., 0.5 mg/kg-day.

In another important study, the day of vaginal opening and first estrus was also observed to be significantly earlier in female rats exposed to 25-100 mg/kg-day of methoxychlor beginning on post-partum day 21 (Gray et al., 1989). Estrous cyclicity started earlier in animals exposed to 25 mg/kg-day, at a normal age in animals exposed to 50-100 mg/kg-day, and was delayed in animals dosed with 200 mg/kg-day. The more sensitive of these results are summarized in Table 3. Similarly, precocious vaginal opening and estrus were noted in female rats exposed in utero, through their mother's milk, and/or postweaning to 50-60 mg/kg-day (Harris et al., 1974; Gray et al., 1989).

Table 3. Selected reproductive effects of methoxychlor in female rats (Gray et al., 1989)

EFFECT	**DOSE, mg/kg-d**				
	Control	25	50	100	200
Age at vaginal opening (days)	32	26*	25*	--	--
	34	--	--	26*	25*
	32	--	--	25*	25*
BW at vaginal opening (g)	97	64*	61*	--	--
	116	--	--	70*	65*
	113	--	--	71*	66*
Age at first cycle (days)	34	29*	36	--	--
	35	--	--	39	63*
	36	--	--	42	89*

* $p < 0.01$

Methoxychlor produces gross and histopathological changes in female reproductive tissues after repeated oral treatments. Lipid accumulation was observed in ovarian interstitial and thecal cells of mice exposed to 200 mg/kg-day for four weeks (Martinez and Swartz, 1992). A two to three-fold increase in uterine weight was observed in ovariectomized mice exposed to 16.7 mg/kg-day for three days (Tullner, 1961). This technique is a relatively sensitive assay for estrogenicity; the ability of a chemical to replace natural estrogens in a primed, estrogen-deficient animal is not,

strictly speaking, an adverse effect. Increased vaginal cornification was observed in female rats exposed to 50-200 mg/kg-day for several days beginning on postpartum day 21 (Gray et al., 1989). An enlarged uterus was observed in rats exposed to 150 mg/kg-day for six weeks (Harris et al., 1974) and in pigs exposed to 1,000 mg/kg-day for 24 weeks (Tegeris et al., 1966). Mammary gland hyperplasia was also observed in the pigs. Repeated doses of 50-400 mg/kg-day methoxychlor produced atrophic changes in ovaries of mice and rats similar to those produced by estrogens (Bal, 1984; Gray et al., 1988, 1989; Martinez and Swartz, 1991).

Methoxychlor effects on estrous cyclicity can adversely affect reproductive function and decrease fertility. Female rats exposed to 100 mg/kg-day methoxychlor beginning on post-partum day 21 showed a 40% decrease in fertility and live pups/litter when mated with untreated males, and an 80% decrease when mated with similarly treated males (Gray et al., 1989). Higher doses of methoxychlor (200 mg/kg-day) produced infertility in 100% of the animals; lack of implantation sites indicated that the effect occurred prior to implantation. Infertility was also observed in female rats following intermediate-duration exposure to 100 mg/kg-day (Bal, 1984). Decreased fertility was observed in rats exposed to 50 mg/kg-day for three generations (Haskell Laboratories, 1966; probably same study as du Pont, 1966). No effects on fertility were noted at 10 mg/kg-day methoxychlor. In female rats, a decreased mating frequency and a decreased fertility in those that mated were noted following exposure to 60-150 mg/kg-day (Harris et al., 1974). Increased resorptions have been consistently reported in rats following acute and intermediate-duration exposures to 35.5-200 mg/kg-day methoxychlor (Harris et al., 1974; Culik and Kaplan, 1976; Kincaid Enterprises, 1986; Cummings and Gray, 1989; Gray et al., 1989; Cummings and Perreault, 1990). Acceleration of embryo transport into the uterus appears to be one mechanism responsible for increases in preimplantation loss (Cummings and Perreault, 1990).

Changes in sex hormone levels are also observed after methoxychlor treatments. Decreased serum progesterone levels were observed in female rats at 50-100 mg/kg-day, but not at 25 mg/kg-day (Cummings and Gray, 1989; Cummings and Laskey, 1993). Pituitary levels of prolactin were decreased in intact female rats but increased in ovariectomized rats exposed to 400 mg/kg-day (Gray et al., 1988). Martinez and Swartz (1992) speculated that methoxychlor causes a feedback inhibition of pituitary hormone secretions resulting in a lack of stimulation of ovarian cells to produce their usual hormones, which sustain the function of uterus and other reproductive tissues.

Reproductive Effects in Male Animals

Oral exposure to methoxychlor can produce gross and histopathological changes in the male reproductive system. Decreased testes weight was observed in male rats and mice exposed to 50-1,400 mg/kg-day (Wenda-Rozewicka, 1983; Bal, 1984; Gray et al., 1989; Chapin et al., 1997). Several reproductive organs (testes, epididymis, seminal vesicles, and prostate) exhibited significantly lower weights in male rats exposed through their dams to 50 or 150 mg/kg-day from gestational day 14 to postnatal day 7, then gavaged directly with the same dose through day 46. No effects were observed at the dose of 5 mg/kg-day.

Decreased prostate weight was observed in male rats exposed to 154 mg/kg-day for 90 days (Shain et al., 1977), and a decreased caudal epididymal sperm count was observed in rats exposed to 50-100 mg/kg-day starting at postnatal day 21 (Gray et al., 1989). Estrogenic effects on testes during critical developmental periods are considered as a potential cause of cancer (McLachlan et al., 1998) or reproductive impairments (Toppari et al., 1996).

Exposure to methoxychlor adversely affects reproductive development or function in male animals. Preputial separation was significantly delayed in male rats exposed to 50 or 150 mg/kg-day through their dams from gestational day 14 through postnatal day 7, then directly gavaged from postnatal day 7 through day 42 (Chapin et al., 1997), suggesting that sexual maturity may be delayed. Doses of 100 or 200 mg/kg-day beginning on postpartum day 21 (Gray et al., 1989) similarly delayed preputial separation. Fertility was decreased by 80% when males rats exposed to 100 mg/kg-day were mated with similarly treated females, compared to only a 50% decrease when untreated males were mated with treated females (Gray et al., 1989). Decreased fertility was also reported in male mice treated with 60 mg/kg-day of methoxychlor (Wenda-Rozewicka, 1983) and in male rats at 150 mg/kg-day in the studies of Chapin et al. (1997). In addition, mating frequency and fertility in male rats that mated were significantly reduced after exposure to methoxychlor in utero, during lactation, and/or postweaning at 60 mg/kg-day (Harris et al., 1974).

Methoxychlor also produces changes in hormone levels in male animals. Exposure to 25-50 mg/kg-day methoxychlor increased levels of prolactin, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) in the pituitary of male rats (Goldman et al., 1986; Gray et al., 1989). Serum levels of TSH, testosterone, and progesterone were decreased in rats dosed with 100 mg/kg-day of methoxychlor (Cummings and Gray, 1989; Gray et al., 1989). Similarly, reduced levels of testosterone were reported in the interstitial fluid and epididymis of male rats exposed to 100 mg/kg-day (Gray et al., 1989).

Immunotoxicity

No specific information is available on immunotoxicity of methoxychlor.

Neurotoxicity

Large doses of methoxychlor, 2,500 mg/kg or more administered orally to rats, decreased locomotor activity and caused tremors (Cannon Laboratories, 1976). In dogs, 1,000-4,000 mg/kg-day orally for 8-24 weeks produced dose-dependent apprehension, nervousness, increased salivation, tremors, convulsions, and death (Tegeris et al., 1966). Inhibiting metabolism of methoxychlor appeared to increase the acute tremors, suggesting that this effect is due to the intact chemical. This is consistent with the observation that DDT, which is a close structural analogue, but very slowly metabolized, produces similar neurological signs (ATSDR, 1994). An increased incidence of hunched posture and rough fur was reported in rats exposed to 22-69 mg/kg-day methoxychlor in feed for 78 weeks (NCI, 1978). No changes in brain weight or histopathology were noted in rats or mice chronically exposed to 69 and 454 mg/kg/day methoxychlor, respectively (NCI, 1978).

Exposure to methoxychlor has also produced behavioral changes in animals consistent with its estrogenic actions (Gray et al., 1988), such as increased wheel-running activity and receptivity to mating. These do not appear to represent neurotoxic effects.

Chronic Toxicity

Several chronic studies have been carried out on methoxychlor, although none of these meet current U.S. EPA guidelines (Haag et al., 1950; Hodge et al., 1952; Deichmann et al., 1967; NCI, 1978). Decreased body weight gain was observed in rats at 69 mg/kg-day (NCI, 1978) or 125 mg/kg-day (Haag et al., 1950) and in mice at 454 mg/kg-day (NCI, 1978).

Unlike many other polycyclic aromatic hydrocarbons, little liver enzyme induction occurs with chronic exposures to methoxychlor, apparently because of its rapid metabolism. Multiple daily doses of methoxychlor can produce significant liver enzyme induction, however, which will further increase its cytochrome P_{450}-mediated metabolism (Li et al., 1995). Traditional microsomal enzyme inducers such as phenobarbital can also induce methoxychlor metabolism (Stresser et al., 1996).

The most significant effects of repeated doses of methoxychlor are on reproductive tissues. Chronic effects can occur after subacute exposures to methoxychlor during critical developmental stages. Perinatal administration of moderate doses of methoxychlor causes persistent stimulation of ovarian/uterine development, well into adulthood. High doses inhibit development, which is similar to the effects of other estrogens according to Eroschenko et al. (1995). Alteration of adult behaviors in male mice by prenatal exposures to methoxychlor and other estrogenic chemicals has also been reported (vom Saal et al., 1995). Administration of a few high doses of methoxychlor to female mice during one pregnancy also appeared to alter the vaginal development of female offspring in a subsequent pregnancy (Swartz and Corkern, 1992).

Chronic administration of methoxychlor disrupts sex-hormone sensitive systems by direct action of the metabolites on the end organs as well as affects on the feedback loops (Cummings, 1997). Uterine weights increase because the uterus responds directly to estrogens (Tullner, 1961), while testicular weights decrease (Hodge et al., 1950; Bal, 1984), presumably because of indirect effects of the metabolites on androgenic tissues. Subchronic or chronic administration of methoxychlor will thus impair reproduction in both males and females (Harris et al., 1974; Bal, 1984; Cummings and Gray, 1989; Gray et al., 1989). Chronic administration does not result in accumulation of methoxychlor or its metabolites, nor in enhanced toxicity compared to subacute administration during critical developmental periods.

Carcinogenicity

Carcinogenicity studies on methoxychlor are inadequate by present standards (such as U.S. EPA guidelines and NTP protocols), but show little evidence of effects. Hodge et al. (1952) reported no significant increase in tumors in rats at daily oral doses up to 80 mg/kg for two years. Deichmann et al. (1967) found no increase in tumors in rats at 50 mg/kg-day in feed for two years. The NCI (1978) also reported that methoxychlor was not carcinogenic in rats fed up to 69 mg/kg-day and in mice fed up to 454 mg/kg-day for 78 weeks. However, Reuber evaluated several chronic studies, including some unpublished FDA data, and concluded that methoxychlor is carcinogenic. His judgment was that methoxychlor produced liver tumors in mice, rats, and possibly dogs, testicular tumors in male mice, bone cancer in female mice, and ovarian tumors in female rats (Reuber, 1979a,b, 1980). The U.S. EPA (1987b) reevaluated these data and

concluded that Reuber's analyses involved inappropriate use of control data and questionable histopathological interpretations. Both the U.S. EPA and the International Agency for Research on Cancer (IARC) have subsequently judged methoxychlor to be not classifiable as to human carcinogenicity (U.S. EPA, 1998; IARC, 1987).

Further evidence on potential carcinogenicity of methoxychlor includes some positive mutagenicity results (Mitchell et al., 1988; Oberley et al., 1993), formation of reactive intermediates which produce covalently bound protein adducts (Bulger et al., 1983), structure-activity correlations based on the carcinogenicity of related weakly estrogenic compounds such as DDT and DDE, and evidence that estrogens are risk factors in testicular tumors (McLachlan, 1998). Metabolic cooperation in Chinese hamster cells was inhibited by methoxychlor. This was similar to the effects of DDT and its analogues, and is a possible indicator of promoter activity (Kurata et al., 1982). However, dermal administration of methoxychlor neither induces nor promotes the formation of skin tumors (Dwivedi and Tabbert, 1994).

Toxicological Effects in Humans

Acute Toxicity

Two reports of toxic effects in humans acutely exposed to methoxychlor are available, although both involve single cases, and exposure to pesticide mixtures. Zeim (1982) reported delayed adverse effects in a 49-year-old male exposed by inhalation to a mixture of methoxychlor and captan (a fungicide often used in mixtures with insecticides on fruit trees). The subject died six months after the exposure due to aplastic anemia. Exposure levels are unknown and the relationship of the effect to the methoxychlor exposure is uncertain.

Harell et al. (1978) reported neurological effects after exposure for 15 to 20 minutes to a pesticide mixture containing 15% methoxychlor and 7.5% malathion. The 21-year old male noted blurred vision and nausea 8-9 hours after exposure, followed by severe abdominal cramps and diarrhea that required hospital admission 36 hours after exposure, followed by dizziness and complete deafness 4 days later, accompanied by several sensory and motor neurological impairments such as limb paresthesia. These effects persisted for at least six years. The authors postulated that the effects could be due to a deficiency of a malathion-metabolizing enzyme. There are no other reports of such effects from malathion, methoxychlor, or a mixture of the two, so the relationship of the effects to methoxychlor exposure is unknown.

Subchronic Toxicity

A single study of subchronic administration of methoxychlor has been conducted in humans. In this study, oral doses of 2 mg/kg-day of methoxychlor (the only dose level studied) for seven days/week for six weeks had no reported adverse effects in either men or women (Stein, 1968; Coulston and Serrone, 1969). Blood studies and bone marrow and liver biopsies revealed no changes attributable to methoxychlor. Two mg/kg-day was considered to be a NOAEL (ATSDR, 1994).

Genetic Toxicity

Methoxychlor was found not to increase single-stranded DNA breaks in human (or rat) testicular cells in vitro, in an analysis of the ability of several chemicals to induce genetic damage (Bjorge et al., 1996).

Developmental and Reproductive Toxicity

Effects of methoxychlor on estrogen receptors were shown to be similar in experimental animals and human tissues (Shelby et al., 1996). In a yeast human estrogen receptor assay, methoxychlor was active, which was inferred as evidence for the metabolism of methoxychlor to the phenolic metabolite as well as competence in binding of the metabolite to the human receptor (Odum et al., 1997; Gaido et al., 1997). Histopathologic changes were not found in the testes of men experimentally exposed for 6 weeks to up to 2 mg/kg of methoxychlor, nor were there changes in menstrual cycles of women after the same dosing regimen (Stein, 1968; Coulston and Serrone, 1969). By analogy with effects observed in animals and effects caused by other estrogenic chemicals such as diethylstilbestrol, it is likely that effects would be observed at lower doses if humans were treated during a more sensitive period, such as during the development and maturation of reproductive organs (see Chapin et al., 1997).

Immunotoxicity

No information is available on immunological effects of methoxychlor in humans.

Neurotoxicity

The acute excitatory effect of methoxychlor associated with DDT-like stimulation at sodium channels should be applicable to humans. However, no reports of human exposures to high doses where this effect would be expected are available. The only report concerning potential neurotoxic effects of methoxychlor is the report of Harrell et al. (1978) mentioned above, in which persistent deafness and severe neurological changes occurred in an adult male beginning several days after a single exposure to a mixture of methoxychlor and malathion. The relationship of methoxychlor to these effects is not known.

Chronic Toxicity/Carcinogenicity

An epidemiological study of men in Minnesota and Iowa suggested an association between leukemia and farming (Brown et al., 1990). In this study, there were positive correlations with odds ratios (ORs) of 2.0 or more for exposure to several agricultural pesticides including three organophosphates (adjusted OR 2.0 to 11.1), pyrethrins (OR 3.7), and methoxychlor (OR 2.2). For methoxychlor, this represented 11 cases of leukemia among 578 farmers with occupational exposure to methoxychlor versus 16 cases out of 1,245 controls with no known exposure. The

statistically significant OR of 2.2 for methoxychlor incorporated adjustments for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high risk occupations and high risk exposures. However, firm conclusions as to a relationship between methoxychlor and leukemia are not possible on the basis of this single study with multiple exposures and risk factors.

No studies are available pertinent to other possible effects of chronic methoxychlor exposure, including the presumed critical effect, endocrine system changes. The widely discussed potential effects of environmental estrogens on reproductive function (Toppari et al., 1996; U.S. EPA, 1997; Safe et al., 1998; Cheek et al., 1998) cannot be specifically associated with methoxychlor because of its short biological half-life and low or undetectable concentrations in the environment.

DOSE-RESPONSE ASSESSMENT

The lowest-dose effects were observed in experimental animals on the reproductive system when treatments were administered during the perinatal period. Chapin et al. (1997) observed several developmental and reproductive system effects in female rats exposed to methoxychlor through their mothers plus direct gavage treatments beginning at postnatal day 7. LOAELs for delayed vaginal opening and decreased ovary weight at postnatal day 46 were 5 mg/kg, the lowest dose tested. Decreased weight of the empty uterus at day 18 of pregnancy and lowered FSH levels during estrus were also observed in adult female rats after perinatal treatments with methoxychlor at doses as low as 5 mg/kg in the Chapin et al. (1997) study. The dose-response for these critical effects was shown above in Table 2 and Figures 3 to 5.

Other developmental toxicity studies have shown adverse effects during critical developmental stages at the 25 to 35 mg/kg-day range, with NOAELs at 5 to 10 mg/kg-day (rat, du Pont, 1976; rabbits, Kincaid Enterprises, 1986; rats, Gray et al., 1989). Several reproductive or developmental effects of methoxychlor have also been observed in experimental animals at the 50 to 100 mg/kg-day dose range in subchronic studies. More significant or more severe effects are demonstrated at the higher exposure levels.

In chronic studies LOAELs of 69 mg/kg-day (rats, NCI, 1978) or more (rats, Haag et al., 1950; mice, NCI, 1978) have been reported, with decreased body weight gain being the critical effect. Chronic NOELs or NOAELs are in the 5 to 10 mg/kg-day range (du Pont, 1951, 1966; Hodge et al., 1952).

In the 1951 du Pont study, methoxychlor was chronically administered to rats in their diet. A systemic NOEL of 100 ppm (5 mg/kg-day) is reported for this study in U.S. EPA 1998, although the critical effect is not mentioned (EPA's review of this unpublished study was requested under FOI, but has not yet been received). The 1996 du Pont report was on a 3-year rat reproduction study (review also not yet received from U.S. EPA). Summaries of this study in U.S. EPA 1998 are similar to those on the Haskell Laboratories 1966 report in ATSDR 1994, and probably both refer to the same study. In the du Pont study, dietary concentrations of 0, 200, or 1000 ppm (0, 10, or 50 mg/kg-day) methoxychlor were administered to ChR-CD rats. Fertility was reduced at 50 mg/kg, accompanied by reduced litter size and reduced fetal viability index. The 10 mg/kg-day dose appeared to slightly reduce food consumption, but was considered to be a NOEL by U.S. EPA (U.S. EPA, 1998). The 1976 du Pont report was on a rat teratology study in which dietary levels of 0, 200, 500, or 1250 ppm (0, 10, 25, or 62.5 mg/kg-day) of methoxychlor were

administered to ChR-CD rats on gestation days 6 through 15. Reduced maternal body weight gain and food consumption, increased post-implantation loss, and a decreased number of live fetuses were observed at the two higher doses. The 10 mg/kg-day dose appears to be a NOAEL, as was also concluded by U.S. EPA (1998). Hodge et al. (1952) conducted a chronic oral toxicity study of methoxychlor in rats. Groups of 25 male and female rats were fed 0, 25, 200, or 1600 ppm (1.25, 10, or 80 mg/kg-day) of methoxychlor for 2 years. Growth retardation occurred in both sexes at 80 mg/kg-day. There were a "large number of tumors" in the 80 mg/kg-day females, but no indication of a significant increase in any specific type (Hodge et al., 1952). The 10 mg/kg-day dose appears to be a NOEL in this study (U.S. EPA, 1998).

U.S. EPA developed its RfD for methoxychlor based upon disruption of reproduction (excessive loss of litters) in a rabbit teratology study. This study had a LOAEL of 35.5 mg/kg-day and a NOAEL of 5.01 mg/kg-day (Kincaid Enterprises, 1986) as reviewed in U.S. EPA 1987c and in the IRIS data base (RfD last updated 8/01/91, U.S. EPA, 1998). EPA applied an uncertainty factor of 1000 to this value, which included factors of 10 for inter- and intra-species differences and an additional factor of 10 "to account for the poor quality of the critical study and for the incompleteness of the data base on chronic toxicity." This resulted in an RfD of 0.005 mg/kg-day with a confidence assessment of "Low." OEHHA concurs with this assessment of the study.

ATSDR (1994) estimated an acute oral minimal risk level (MRL) of methoxychlor of 0.02 mg/kg-day based on the LOAEL of 25 mg/kg-day (lowest dose tested) for accelerated onset of puberty in immature female rats (Gray et al., 1989). ATSDR based an intermediate-duration oral MRL of 0.02 mg/kg-day on the LOAEL of 25 mg/kg-day for elevated levels of prolactin in the pituitary of male rats from the same study (Gray et al., 1989). ATSDR derived no chronic MRL because no sensitive indicators of reproductive function were evaluated in the available chronic studies, and the LOAELs observed in chronic studies were higher than the LOAELs for shorter-duration studies. For estimation of the MRL, they divided the above values by an uncertainty factor of 1000. This was comprised of factors of 10 each for extrapolation to humans from an animal study, variation in sensitivity among humans, and extrapolation from a LOAEL to a NOAEL. The resulting value was rounded from 0.025 to 0.02 mg/kg-day. OEHHA agrees with 25 mg/kg-day as a LOAEL in this study based upon the effects in both male and female rats. However, we believe that the study of Chapin et al. (1997) is more appropriate on which to base the risk assessment because of the lower-dose effect, probably related to the earlier onset of dosing in the Chapin et al. study.

The single subchronic human study (Stein, 1968; Coulston and Serrone, 1969) showed no adverse effects, with an apparent NOAEL of two mg/kg-day (the only dose tested), but did not include treatment during potential sensitive developmental periods (as identified in animal tests). If the safe level were estimated from this study, the known developmental sensitivity to endocrine effects must be accounted for, as well as other sources of human variability (genetic differences, illnesses, dietary effects, etc.). A factor of 10 for each of the two major sources of uncertainty might be appropriate, which would result in an estimated safe dose of about 0.02 mg/kg-day. However, a study in which no adverse effects were found provides a weaker basis for extrapolation than the animal studies, which clearly demonstrate the dose-response for adverse effects on reproductive endpoints and a greater sensitivity during the critical period for development of reproductive organs. Thus this human study is not used as the basis for the PHG.

The Chapin et al. (1997) study utilizing perinatal exposures in rats demonstrates the lowest observed toxic effect level, with an experimental animal LOAEL of 5 mg/kg-day. These data are basically consistent with other studies showing LOAELs of about 25 mg/kg-day following later postnatal treatments, which appears to avoid the most sensitive period (Chapin et al., 1997). Estimation of a safe level for human exposure from these studies under current risk assessment guidelines considers cross-species extrapolation; variability among humans, including potential sensitive subpopulations; and potential extra factors for uncertainty in the database. Severity-of-endpoint and other relevant factors such as exposure to and interactions with other similarly acting chemicals are also considered. Using the lowest LOAEL of 5 mg/kg-day, we applied an uncertainty factor of 10 for cross-species extrapolation, 10 for human variability, and 10 for extrapolation from a LOAEL to a NOAEL, for a combined uncertainty factor of 1000. This combined uncertainty factor should be large enough to account for any sensitive human populations and potential interactions with other estrogenic chemicals. The estimated safe dose is therefore 0.005 mg/kg-day.

The PHG is derived from the LOAEL of 5 mg/kg-day for developmental effects on reproductive system parameters in an animal study, supported by several other animal toxicity studies with similar endpoints.

CALCULATION OF PHG

Calculation of concentrations of chemical contaminants in drinking water associated with negligible risks must take into account the toxicity of the chemical and the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering, flushing toilets, washing dishes and clothes, and other household uses that may result in dermal and inhalation exposures to chemical contaminants. However, because methoxychlor is non-volatile, secondary inhalation exposures are expected to be negligible.

Other sources of exposure to methoxychlor include food and possible occupational or household uses. Although methoxychlor is no longer registered as a pesticide in California, it is still used in other states. It has recently been detected more often in food samples than in drinking water supplies. We consider food to represent the largest likely exposure medium. A default relative source contribution of 20% for drinking water is therefore used in the determination of the PHG.

Calculation of a public health-protective concentration (C, in mg/L) for methoxychlor in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times L/day}$$

where,

NOAEL/LOAEL = No-observed-adverse-effect-level or lowest-observed-adverse-effect-level

BW	=	Adult body weight (a default of 70 kg for male or 60 kg for female; female weight used in this case because effects are found in females)
RSC	=	Relative source contribution (a default of 20% to 80%)
UF	=	Uncertainty factors (often factors of 10 defaults to account for various sources of uncertainty)
L/day	=	Adult daily water consumption rate (default of 2 L/day)

In this case,

$$C = \frac{5 \text{ mg/kg-day} \times 60 \text{ kg} \times 0.2}{1000 \times 2 \text{ L/day}} = 0.03 \text{ mg/L} = \mathbf{30\ ppb}$$

A PHG of 0.03 mg/L (30 ppb) is therefore derived for methoxychlor in drinking water based on the LOAEL of 5 mg/kg-day for developmental effects on the female rat reproductive system (delayed vaginal opening, decreased ovary weight, and decreased weight of the pregnant uterus), which provided an estimated safe dose for human females of 0.005 mg/kg-day.

RISK CHARACTERIZATION

Exposure to multiple estrogenic chemicals in our environment has been a matter of much recent discussion and concern (Shelby et al., 1996; Toppari et al., 1996; U.S. EPA, 1997; DeRosa et al., 1998; Safe, 1998; Cheek et al., 1998). Methoxychlor has often been mentioned in publications on the subject, although there does not seem to be evidence of environmental persistence or bioaccumulation of this chemical (ATSDR, 1994). Recent studies make clear that methoxychlor, mainly through its demethylated metabolites, can interact with human estrogenic receptors (Cummins, 1997; Danzo, 1997, Gaido et al., 1997). Thus it has the potential to become a problem, although its short environmental and in vivo half-life will minimize both exposure and effects, compared to other structurally similar halogenated hydrocarbons.

The primary source of uncertainty in developing the PHG for methoxychlor in drinking water is the question of human sensitivity to the potential endocrine-disruptive effects. Because the endocrine systems are feedback-regulated, exposures to small amounts of estrogenic chemicals may simply be accommodated by normal homeostatic mechanisms (U.S. EPA, 1997). Studies are inadequate to fully characterize sensitive developmental periods and corresponding effective estrogenic doses and concentrations in humans. Therefore it is not clear what dose-equivalent of an estrogenic chemical would be physiologically significant, particularly in the presence of other environmental estrogens (in food or water) (U.S. EPA, 1997; Safe, 1998; Cheek et al., 1998). However, the animal studies appear adequate to document the effects, mechanism of action, and potency of methoxychlor in animals, including the potential for sensitive developmental periods. This is taken into account in the choice of the critical study (Chapin et al., 1997) and application of uncertainty factors. Effects are likely to be additive or competitive among different chemicals

with estrogenic activity. Some reported synergistic interactions among environmental estrogens (Arnold et al., 1996) have not been substantiated by other work (Ramamoorthy et al., 1997; McLachlan, 1997). Interactions of other types of environmental chemicals have also not been remarkable (Heindel et al., 1994; Ito et al., 1995).

While methoxychlor residues are found in foods, an appropriate relative source contribution (RSC) to account for the potential multiple exposure routes is uncertain. We have used the default 0.2 in lieu of specific guidelines on how to calculate RSC for these conditions. OEHHA believes that the PHG level of 0.03 mg/L (30 ppb) is adequate and appropriate to protect humans against adverse effects of methoxychlor in drinking water.

OTHER REGULATORY STANDARDS

U.S. EPA's MCL and MCLG for methoxychlor are both 0.04 mg/L, as currently listed in IRIS (U.S. EPA, 1998) and originally finalized in 1991 (56 FR 3526, 01/30/91). The U.S. EPA's RfD is 0.005 mg/kg-day. The California MCL is also 0.04 mg/L. Methoxychlor is not listed as a developmental or reproductive toxicant under California's Proposition 65. The ambient water quality criterion for human health is 0.1 mg/L (U.S. EPA, 1976). The ambient water quality criterion for aquatic organisms is 3×10^{-5} mg/L (U.S. EPA, 1976, 1998). ATSDR's acute and subchronic oral maximum residue levels are 0.02 mg/kg-day (ATSDR, 1994).

REFERENCES

Appel RJ, Eroschenko VP. (1992) Passage of methoxychlor in milk and reproductive organs of nursing female mice; 1. Light and scanning electron microscopic observations. Reprod Toxicol 6(3):223-31.

Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ, McLachlan JA. (1996) Synergistic interactions of estrogen receptor with combinations of environmental chemicals. Science 272:1489-92.

ATSDR (1994) Toxicological profile for methoxychlor. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA. TP-93/11.

Bal HS. (1984) Effect of methoxychlor on reproductive systems of the rat. Proc Soc Exp Biol Med 176:187-96.

Bjorge C, Brunborg G, Wiger R, Holme JA, Scholz T, Dybing E, Soderlund EJ. (1996) A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod Toxicol 10(6):509-19.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. (1990) Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50(20):6585-91.

Bulger WH, Feil VJ, Kupfer D. (1985) Role of hepatic monooxygenases in generating estrogenic metabolites from methoxychlor and from its identified contaminants. Mol Pharmacol 27:115-24.

Bulger WH, Temple JE, Kupfer D. (1983) Covalent binding of [^{14}C]methoxychlor metabolite(s) to rat liver microsomal components. Toxicol Appl Pharmacol 68(3):367-74.

Bulger WH, Kupfer D. (1990) Studies on the formation of methoxychlor-protein adduct in rat and human liver microsomes. Is demethylation of methoxychlor essential for cytochrome P450 catalyzed covalent binding? Biochem Pharmacol 40(5):937-45.

Cannon Laboratories. (1976) Acute oral toxicity in rats: Technical methoxychlor. Reading, PA. Unpublished study cited in ATSDR, 1994.

CCR (1998) California Code of Regulations Title 22, Article 5.5 Primary Standards – Organic Chemicals, Section 65555.

Chapin RE, Harris MW, Davis BJ, Ward SM, Wilson RE, Mauney MA, Lockhart AC, Smialowicz RJ, Moser VC, Burka LT, Collins BJ. (1997) The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. Fund Appl Toxicol 40(1):138-57.

Cheek AO, Vonier PM, Oberdorster E, Burow BC, McLachlan JA. (1998) Environmental signaling: a biological context for endocrine disruption. Environ Health Perspect 106, Suppl 1:5-10.

Coulston F, Serrone DM. (1969) The comparative approach to the role of nonhuman primates in evaluation of drug toxicity in man: a review. Ann NY Acad Sci 162:681-704.

Culik R, Kaplan AM. (1976) Teratogenic study in rats with ethane, 1,1,1-trichloro-2,2-bis(paramethoxyphenyl) (methoxychlor). Haskell Laboratory Report No. 648-76. Unpublished study cited in ATSDR, 1994.

Cummings AM. (1997) Methoxychlor as a model for environmental estrogens. Crit Rev Toxicol 27(4):367-79.

Cummings AM, Gray LE. (1989) Antifertility effect of methoxychlor in female rats: Dose and time-dependent blockade of pregnancy. Toxicol Appl Pharmacol 97:454-62.

Cummings AM, Laskey J. (1993) Effect of methoxychlor on ovarian steroidogenesis: role in early pregnancy loss. Reprod Toxicol 7:17-23.

Cummings AM, Perreault SD. (1990) Methoxychlor accelerates embryo transport through the rat reproductive tract. Toxicol Appl Pharmacol 102:110-6.

Davison KL, Lamoureux CH, Feil VJ. (1983) Methoxychlor metabolism in goats. 2. Metabolites in bile and movement through skin. J Agric Food Chem 31(1):164-6.

Dehal SS, Kupfer D. (1994) Metabolism of the proestrogenic pesticide methoxychlor by hepatic P450 monooxygenases in rats and humans. Dual pathways involving novel ortho ring hydroxylation by CYP2B. Drug Metab Dispos 22(6):937-46.

Deichmann WB, Keplinger M, Salla F, Glass E. (1967) Synergism among oral carcinogens IV. The simultaneous feeding of four tumorigens to rats. Toxicol Appl Pharmacol 11:88-103.

DeRosa C, Richter P, Pohl H, Jones DE. (1998) Environmental exposures that affect the endocrine system: public health implications. J Toxicol Environ Health, Part B, 1:3-26.

DPR (1996a) Annual pesticide use report, 1995. California Department of Pesticide Regulation, Cal/EPA, December 20, 1996.

DPR (1996b) Residues in fresh produce – 1994. California Department of Pesticide Regulation, Cal/EPA, Sacramento, CA.

Dunkel VC, Pienta RJ, Sivak A, Traul KA. (1981) Comparative neoplastic transformation responses of BALB/3T3 cells, Syrian hamster embryo cells and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens. J Natl Cancer Inst 67:1303-15.

du Pont (1951) EI du Pont de Nemours and Company, Inc., Study MRID No. 00029282, as cited by U.S. EPA (IRIS) Methoxychlor, 1998.

du Pont (1966) EI du Pont de Nemours and Company, Inc., Study MRID No. 00108732, 00113276, as cited by U.S. EPA (IRIS) Methoxychlor, 1998 (possibly same as Haskell Laboratories, 1966).

du Pont (1976) EI du Pont de Nemours and Company, Inc., Study MRID No. 00062704, as cited by U.S. EPA (IRIS) Methoxychlor, 1998.

Dwivedi C, Tabbert J. (1994) Effects of methoxychlor on skin tumor development. Toxicol Lett 74(3):235-40.

Eroschenko VP, Abuel-Atta AA, Grober MS. (1995) Neonatal exposures to technical methoxychlor alters ovaries in adult mice. Reprod Toxicol 9(4):379-87.

Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ, McDonnell DP. (1997) Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143(1):205-12.

Goldman JM, Cooper RL, Rehnberg GL, Hein JF, McElroy WK, Gray LE, Jr. (1986). Effects of low subchronic doses of methoxychlor on the rat hypothalamic-pituitary reproductive axis. Toxicol Appl Pharmacol 86:474-83.

Golovleva LA, Polyakova AB, Pertsova RN, Finkelshtein ZI. (1984) The fate of methoxychlor in soils and transformation by soil microorganisms. J Environ Sci Health B 19:523-38.

Gray LE Jr, Ostby JS, Ferrell JM, Sigmon ER, Goldman JM. (1988) Methoxychlor induces estrogen-like alterations of behavior and the reproductive tract in the female rat and hamster: Effects on sex behavior, running wheel activity, and uterine morphology. Toxicol Appl Pharmacol 96:525-40.

Gray LE Jr, Ostby J, Ferrell J, Rehnberg G, Linder R, Cooper R, Goldman J, Slott V, Laskey J. (1989) A dose-response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. Fund Appl Toxicol 12:92-108.

Gunderson E. (1988) Chemical contaminants monitoring: FDA total diet study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. J Assoc Off Anal Chem 71:1200-09.

Haag HB, Finnegan JK, Larson PS, Reise W, Dreyfuss MI. (1950) Comparative chronic toxicity for warm-blooded animals of 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and 2,2-bis-(p-methoxyphenyl)-1,1,1-trichloroethane (DMDT, methoxychlor). Arch Int Pharmacodyn 83:491-504.

Harell M, Shea JJ, Emmett JR. (1978) Bilateral sudden deafness following combined insecticide poisoning. Laryngoscope 88:1348-51.

Haskell Laboratories. (1966) Three-generation study on rats with 2,2-bis(p-methoxyphenyl)-1,1,1-trichlorethane (methoxychlor). Haskell Laboratory Report No. 102-66. Unpublished study cited in ATSDR, 1994 (probably same study as du Pont, 1966).

Harris SJ, Cecil HC, Bitman J. (1974) Effect of several dietary levels of technical methoxychlor on reproduction in rats. J Agric Food Chem 22:969-73.

Heindel JJ, Chapin RE, Gulati DK, George JD, Price CJ, Marr MC, Myers CB, Barnes LH, Fail PA, Grizzle TB, Schwetz BA, Yang RSH. (1994) Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. Fund Appl Toxicol 22:605-21.

Hodge HC, Maynard EA, Blanchet HJ. (1952) Chronic oral toxicity tests of methoxychlor (2,2-di-(p-methoxyphenyl)-1,1,1-trichloroethane) in rats and dogs. J Pharmacol Exp Ther 104:60-6.

Hoff RM, Muir DC, Grift NP. (1992) Annual cycle of polychlorinated biphenyls and organohalogen pesticides in air in Southern Ontario. 1. Air concentration data. Environ. Sci Technol 26:266-75.

Howard PH, ed. (1991) Handbook of environmental fate and exposure data for organic chemicals. Chelsea, MI: Lewis Publishers, Inc. p. 502-4.

IARC. (1979) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 20: Methoxychlor. International Agency for Research on Cancer, World Health Organization, Lyon, France.

IARC. (1987) IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Suppl. 7 International Agency for Research on Cancer, World Health Organization, Lyon, France.

Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A, Shirai T. (1995) Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Fd Chem Toxic 33(2):159-63.

Ivey MC, Ivie GW, Coppock CE, Clark KJ. (1983) Methoxychlor residues in milk of cattle treated with Marlate 50 insecticide as a dermal spray. J Dairy Sci 66(4):943-50.

Kapoor IP, Metcalf RL, Nystrom RF, et al. (1970) Comparative metabolism of methoxychlor, methiochlor and DDT in mouse, insects, and in a model ecosystem. J Agric Food Chem 18:1145-52.

Kincaid Enterprises. (1986) Rabbit teratology study with methoxychlor, technical grade. Nitro, WV. MRID No. 0015992. Unpublished study cited in U.S. EPA, 1987c, ATSDR, 1994, and U.S. EPA, 1998.

Kupfer D, Bulger WH. (1987) Metabolic activation of pesticides with proestrogenic activity. Fed Proc 46(5):1864-9.

Kupfer D, Bulger WH, Theoharides AD. (1990) Metabolism of methoxychlor by hepatic P-450 monooxygenases in rat and human. 1. Characterization of a novel catechol metabolite. Chem Res Toxicol 3(1):8-16.

Kurata M, Hirose K, Umeda M. (1982) Inhibition of metabolic cooperation in Chinese hamster cells by organochlorine pesticides. Gann 73(2):217-21.

Li HC, Dehal SS, Kupfer D. (1995) Induction of the hepatic CYP2B and CYP3A enzymes by the proestrogenic pesticide methoxychlor and by DDT in the rat. Effects on methoxychlor metabolism. J Biochem Toxicol 10(1):51-61.

McLachlan JA. (1997) Synergistic effect of environmental estrogens: report withdrawn. Science 277:462-3.

McLachlan JA, Newbold RR, Li S, Negishi M. (1998) Are estrogens carcinogenic during development of the testes? APMIS 106(1):240-2.

Martinez EM, Swartz WJ. (1991) Effects of methoxychlor on the reproductive system of the adult female mouse. 2. Gross and histologic observations. Reprod Toxicol 5:139-47.

Martinez EM, Swartz WJ. (1992) Effects of methoxychlor on the reproductive system of the adult female mouse. 2. Ultrastructural observations. Reprod Toxicol 6:93-8.

Mitchell AD, Rudd CJ, Caspary WJ. (1988) Evaluation of L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at SRI International. Environ Mol Mutagen 12:37-101.

Muir DC, Yarechewski AL. (1984) Degradation of methoxychlor in sediments under various redox conditions. J Environ Sci Health 3:271-95.

Myhr BC, Caspary WJ. (1988) Evaluation of L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. Environ Mol Mutagen 12:103-94.

NCI. (1978) Bioassay of methoxychlor for possible carcinogenicity. National Cancer Institute, Dept. of Health, Education and Welfare, Washington, DC. Technical Report Series No. 35.

Oberly TJ, Michaelis KC, Rexroat MA, Bewsey BJ, Garriott ML. (1993) A comparison of the CHO/HGPRT+ and the L5178Y/TK+/- mutation assays using suspension treatment and soft agar cloning: results for 10 chemicals. Cell Biol Toxicol 9(3):243-57.

Odom J, Lefevre PA, Tittensor S, Paton D, Routledge EJ, Beresford NA, Sumpter JP, Ashby J. (1997) The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Regul Toxicol Pharmacol 25(2):176-88.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ Mut 3:11-32.

Ramamoorthy K, Wang F, Chen I-C, Safe S, Norris JD, McDonnell DP, Gaido KW, Bocchinfuso WP, Korach KS. (1997) Potency of combined estrogenic pesticides. Science 275:405.

Reuber MD. (1979a) Carcinomas of the liver in Osborne-Mendel rats ingesting methoxychlor. Life Sci 24:1367-72.

Reuber MD. (1979b) Interstitial cell carcinomas of the testis in balb/C male mice ingesting methoxychlor. Cancer Res Clin Oncol 93:173-9.

Reuber MD. (1980) Carcinogenicity and toxicity of methoxychlor. Environ Health Perspect 36:205-19.

Safe S. (1998) Interactions between hormones and chemicals in breast cancer. Ann Rev Pharmacol Toxicol 38:121-58.

Sax NI. (1987) Methoxychlor. In: Dangerous Properties of Industrial Materials. New York: Van Nostrand Reinhold. Suppl. Sept/Oct 1987, pp. 79-87.

Shain SA, Shaeffer JC, Boesel RW. (1977) The effect of chronic ingestion of selected pesticides upon rat ventral prostate homeostasis. Toxicol Appl Pharmacol 40:115-30.

Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. (1996) Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect 104(12):1296-300.

Simmon VF. (1979) In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of 18 pesticides. EPA 600/1-79-041.

Skaare JU, Berge G, Odegaard S, Grave K. (1982) Excretion of methoxychlor in cow milk following dermal application. Acta Vet Scand 23(1):16-23.

Stein AA. (1968) Comparative methoxychlor toxicity in dogs, swine, rats, monkeys, and man. Med Surg 37:540-1.

Stresser DM, Dehal SS, Kupfer D. (1996) Ring hydroxylation of [o-^{3}H]methoxychlor as a probe for liver microsomal CYP2B activity: potential for in vivo CYP2B assay. Anal Biochem 233(1):100-7.

Swartz WJ, Corkern M. (1992) Effects of methoxychlor treatment of pregnant mice on female offspring of the treated and subsequent pregnancies. Reprod Toxicol 6(5):431-7.

Tegeris AS, Earl FL, Smalley HE, et al. (1966) Methoxychlor toxicity: Comparative studies in the dog and the swine. Arch Environ Health 13:776-87.

Toppari J, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkeback NE. (1996) Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104, Suppl 4:741-803.

Traul KA, Takayama K, Kachevsky V, Hink RJ, Wolf JS. (1981) Rapid in vitro assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independent endpoint. 2. Assay validation. J Appl Toxicol 1:190-5.

Tullner WW. (1961) Uterotrophic action of the insecticide methoxychlor. Science 133:647-8.

vom Saal FS, Nagel SC, Palanza P, Boechler M, Parmigiani S, Welshons WV. (1995) Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behaviour in male mice. Toxicol Lett 77:343-50.

U.S. EPA. (1976) Quality criteria for water, July 1976. PB-263943.

U.S. EPA. (1987a) Drinking water criteria document for methoxychlor. Office of Health and Environmental Assessment. PB89-192215.

U.S. EPA. (1987b) Methoxychlor health advisory. Office of Drinking Water. Washington, DC.

U.S. EPA. (1987c) Review of teratology study in rabbits with methoxychlor (Kincaid Enterprises, 1986). Office of Pesticides and Toxic Substances, MRID 005881.

U.S. EPA. (1988) Pesticide Fact Sheet: Methoxychlor. Office of Pesticides and Toxic Substances. EPA 540/FS-89-014.

U.S. EPA. (1990a) Non-occupational pesticide exposure study (NOPES). Office of Research and Development, Washington, DC. EPA 600/3-90/003.

U.S. EPA. (1990b) National Pesticide Survey. Summary results of EPA's national survey of pesticides in drinking water wells. Office of Water, Washington, DC.

U.S. EPA. (1996) Guidelines for Reproductive Toxicity Risk Assessment. Office of Research and Development, Washington, DC. Federal Register 61(212):56273-322, EPA 630/R-96/009a.

U.S. EPA. (1997) Special report on environmental endocrine disruption: an effects assessment and analysis. Prepared by a technical panel for the U.S. EPA Risk Assessment Forum. Office of Research and Development, Washington DC. EPA 630/R-96/012.

U.S. EPA. (1998) Integrated Risk Information System (IRIS): Methoxychlor, oral RfD last revised 8/91, cancer assessment updated 10/90. Online chemical toxicity database, available at www.epa.gov/iris, accessed 3/98.

U.S. FDA. (1996) Pesticide program, residue monitoring 1995. U.S. Food and Drug Administration, Washington, DC.

Wania F, Mackay D. (1996) Tracking the distribution of persistent organic pollutants. Environ Sci Technol 30:390-6.

Waters MD, Sandhu SS, Simmon VF, et al. (1982) Study of pesticide genotoxicity. Basic Life Sci 21:275-326. As cited in U.S. EPA, 1987.

Welch HE, Muir DC, Billeck BN et al. (1991) Brown snow: A long-range transport event in the Canadian arctic. Environ Sci Technol 25:280-6.

Wenda-Rozewicka L. (1983) Morphometric studies of male gonads from mice receiving insecticides (Metox-30, Sadofos-30 and Foschlor-50). Folia Biol 32:23-34.

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S. (1990) Percutaneous absorption of ^{14}C-DDT and ^{14}C-benzo[a]pyrene from soil. Fund Appl Toxicol 15:510-6.

Zhou LX, Dehal SS, Kupfer D, Morrell S, McKenzie BA, Eccleston ED Jr., Holtzman JL. (1995) Cytochrome P450 catalyzed covalent binding of methoxychlor to rat hepatic, microsomal iodothyronine 5'-monodeiodinase, type I: does exposure to methoxychlor disrupt thyroid hormone metabolism? Arch Biochem Biophys 322(2):390-4.

Ziem G. (1982) Aplastic anaemia after methoxychlor exposure. Lancet, December 11, 1349.